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New survey data on abundance and movements for two poorly known Asian Spiny Frogs

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Abstract

We present new natural history data on abundance and movements (daily and seasonal) during the pre-breeding (March-June) and breeding-post-breeding season (July-September) of two poorly studied frog species of the Himalayas, *Nanorana vicina* and *Allopaa hazarensis*. We estimated 185 Murree Hills Frogs and 90 Hazara Frogs within the study area (0.79 ha). The daily and seasonal movement data showed that the two species moved either between neighboring ponds or remained in an array of smaller ponds (within an area of 120 m^2) along the stream bank. About 75% of movements were < 29.5 m in *N. vicina* and < 50.87 m in *A. hazarensis* during pre-breeding season while < 41.5 m in *N. vicina* and < 81 m in *A. hazarensis* during breeding-post-breeding season. We suggest inclusion of amphibian habitat requirements and ensuring stream connectivity in urban planning and development projects in the area to prevent the local extinction of the endemic species. In the future, more robust and long-term studies, encompassing more streams situated within a wider area, would help clarify dispersal, colonization, metapopulation structure, and dynamics of these endemic frogs of the forested montane streams in the Himalayan Foothills.

Key Words

Dicroglossidae, Highland Frogs, Himalaya, Lincoln Index, Paini tribe

Introduction

The temporal and spatial aspects of animal movement are considered important for population control, metapopulation dynamics and life-long sustainability of wildlife species. The impact of land use, the spread of invasive species, diseases, and responses to global climate change, can be better understood if we comprehend animal movement and dispersal (Smith and Green 2006). Movement is essential for the reproduction and survival of local populations and in a wider landscape or regional scale, mobility among populations is necessary for recolonization after local extinction and for the maintenance of metapopulations (Marsh and Trenham 2001). Young amphibians seldom disperse more than 2–3 kilometers (Petranka and Holbrook 2006) and rarely

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move more than five kilometers (Peter 2001). Amphibian dispersal is vital for the maintenance of amphibian populations and yet this is often significantly compromised in anthropogenically impacted landscapes (Ribeiro et al. 2019).

Currently, 21 species of amphibian (all anurans) have been documented in Pakistan (Rais et al. 2021) of which nine are believed to be endemic to Pakistan (Ali et al. 2018). *Allopaa hazarensis*, Family Dicroglossidae, is endemic to the springs and streams of Northern Pakistan (> 1,195 m elevation) while *Nanorana vicina*, Family Dicroglossidae, is endemic to Pakistan (> 1,765 m elevation) and India (Khan 2006; Ahmed et al. 2020) (Fig. 1). The two frog species are listed as Least Concern in IUCN Red List of Threatened Species. The major threats to these frog species include habitat degradation, urbanization, and climate change (Ohler and Dutta 2004; Khan et al. 2008).

The description of their adults and tadpoles is available (Dubois and Khan 1979; Khan and Malik 1979; Rais et al. 2014; Gill et al. 2020; Ikram et al. 2022). Further information about molecular taxonomy and phylogeny (Hofmann et al. 2019, 2021; Akram et al. 2021), impact of climate change (Saeed et al. 2021, 2022a), and occupancy modeling and population monitoring through eDNA (Saeed et al. 2022b) have been carried out recently. The post-breeding radiotelematry (VHF) revealed that the two species show little movement (< 3 m) (Akram et al. 2022). These two frog species breed during summer and monsoon: July-August (Saeed et al. 2021). There is no study available on what triggers them to breed and regarding their clutch size and age structure. The temperature had a positive correlation with increase in the concentrations of sex hormones (Saeed et al. 2021). Since these frogs inhabit forested streams having hard and rocky substrate (Khan 2006), these are believed to lay eggs under the heavy boulders where early development takes place. Under laboratory conditions, *A. hazarensis* completed metamorphosis during 12^{th}-18^{th} week while *N. vicina* completed metamorphosis during 18^{th}-22^{th} week with 22 °C as the most stable temperature for their growth. Although higher temperatures (>26 °C) were associated with early onset and completion of metamorphosis, they caused reduction in the body size, more frequent developmental complications or deformities such as edema and tail kinks, lower fitness and higher mortality (Saeed et al. 2021).

Since not much is known about the natural history of these two Himalayan endemics, we aimed to provide abundance data and examine daily and seasonal movements of the *N. vicina* (Murree Hills Frogs) and the *A. hazarensis* (Hazara Frogs) along a continuous habitat- montane forested freshwater stream. Our findings will help survey anurans associated with similar habitats elsewhere in the world. Our data on relative abundance and movement patterns of the two Himalayan endemics help increase understanding of their habitat requirements, metapopulation structure, dispersal, and colonization.

Materials and methods

Study area system and species

We conducted the study at a permanent freshwater stream (Fig. 2) located in Murree Tehsil, Rawalpindi District, Punjab Province, Pakistan. The study area is a part of the Western Himalayan Ecoregion which is included in Global 200 Ecoregions of Ecological Significance. Murree spreads over an area of about 697.5 km^2 with an elevation of 550–2,600 m (Shahzad et al. 2015). The climate is subtropical highland (Cwb as per Koppen climate classification) (Beck et al. 2018). The mean maximum temperature of the area is 25 °C while the mean annual precipitation is 1,789 mm (EPDP 2010). The precipitation generally occurs in the form of snow during the winter (December, January, and February) and rain during the summer. The study area features subtropical pine forest with Chir Pine (*Pinus roxburghii*) as the dominant plant species of the area (Shahzad et al. 2015).

The study sites consisted of six ponds, connected by a permanent stream at an elevation of between 1,660 and 1,705 m (Fig. 3). It cascades over rapids and the nearest adjacent streams are 370 m and 1,750 m (straight linear distance) away over low ridges. We selected a section of 133 m of the stream and surveyed frogs 30 m on either side of it (0.79 ha.).

Study duration, sampling, tagging, estimation of distance moved and population size

We gathered data from March, 2019, to September, 2019, during pre-breeding (March-June), and breeding-post-breeding seasons (July-September). We performed an eight-day field session in each season. During the first field season (May 17–24, 2019), we captured and marked 74 (33 ♂, mean snout-vent length, SVL: 67.02 ± 11.67; 41 ♀, SVL: 68.02 ± 10.62) individuals of *N. vicina* and 15 (8 ♂, SVL: 54.18 ± 19.15; 7 ♀, SVL: 64.41 ± 24.34) of *A. hazarensis* (Suppl. material 1: table 1a). We measured the straight distance (meters) between the ponds using measured rope to determine the distance between the pond where the frog was released to the pond where it was recaptured. We flagged rocks and trees using pre-measured ropes of different lengths (10 m, 20 m, and 30 m) on either side of the stream to note the distance moved by the specimens away from the stream (Fig. 3). This eliminated the need to measure distance moved by the frogs, manually or through a GPS each time. The survey team consisted of 8–10 observers. The surveyors moved upstream (from first pond to the last identified), capturing adults for tagging and estimating the distance moved. We carried out the surveys 4–5 hours after sunset, collected frogs using a dip net and gathered the data as described. We identified the species

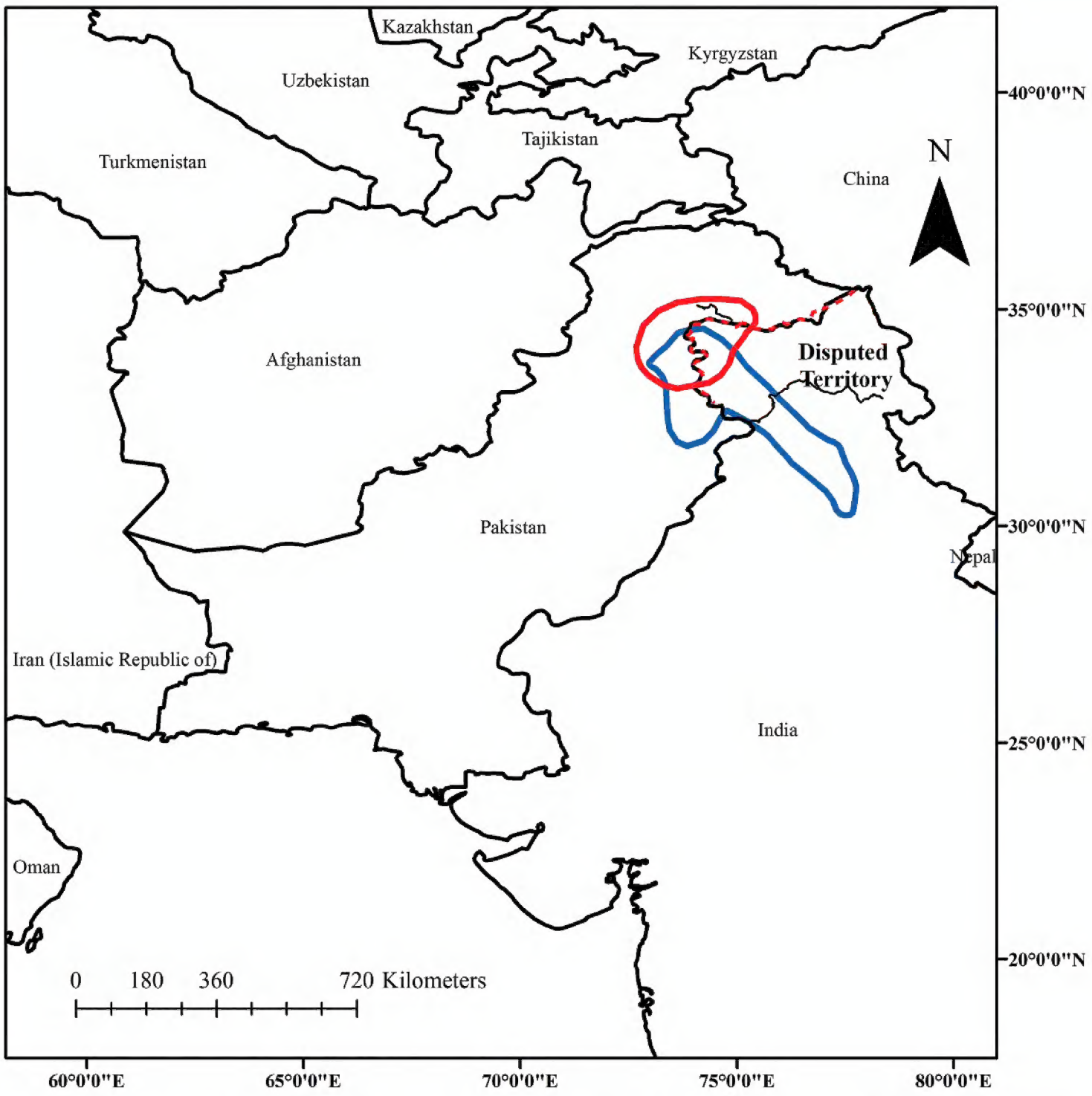

Figure 1. Global distribution range of Murree Hills Frog (*Nanorana vicina*) (blue polygon) and Hazara Frog (*Allopaa hazarensis*) (red polygon).

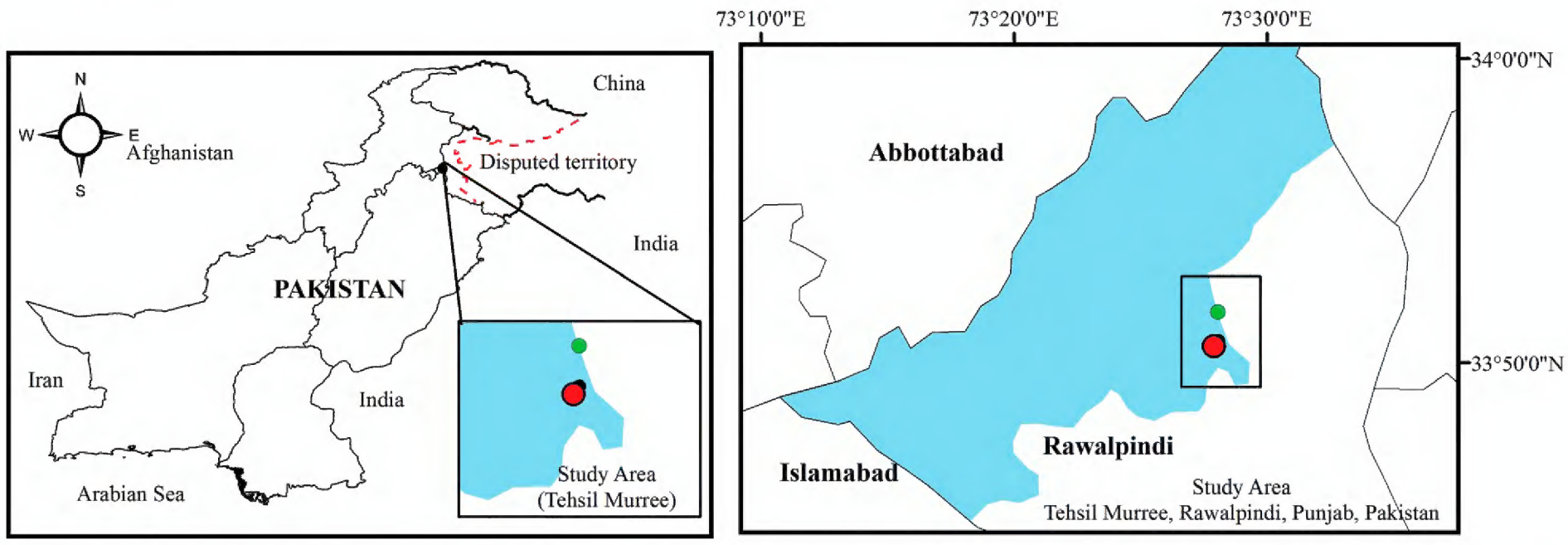

Figure 2. Map showing location of the study area and the study stream in Village Parhanna, Tehsil Rawalpindi, Province Punjab, Pakistan (left) Study stream and other nearby streams shown in red circle 370 m and green circle 1750 m from the study stream (right).

using Khan (2006), their sex (females have soft belly) and measured the snout-vent length (SVL) using a digital vernier caliper (0.01 mm, Insize). We marked the frogs using toe-clipping (Guimaraes et al. 2014; Ginnan et al. 2015), released them back and noted the point of release (pond number). We repeated the procedure for eight consecutive days, and recorded the distance (from the pond where the frog was released to where it was recaptured). We then estimated the mean daily distance (8 days) and distance moved per day. While during the second field season (August 28–September 4, 2019), we captured and marked 10 (5 ♂, SVL: 68.39 ± 30.58; 5 ♀, SVL: 61.11 ± 29.93) *N. vicina* and 30 (16 ♂, SVL: 56.96 ± 14.24; 7 ♀, SVL: 65.59 ± 17.52) *A. hazarensis* (Suppl. material 1: table 1b)

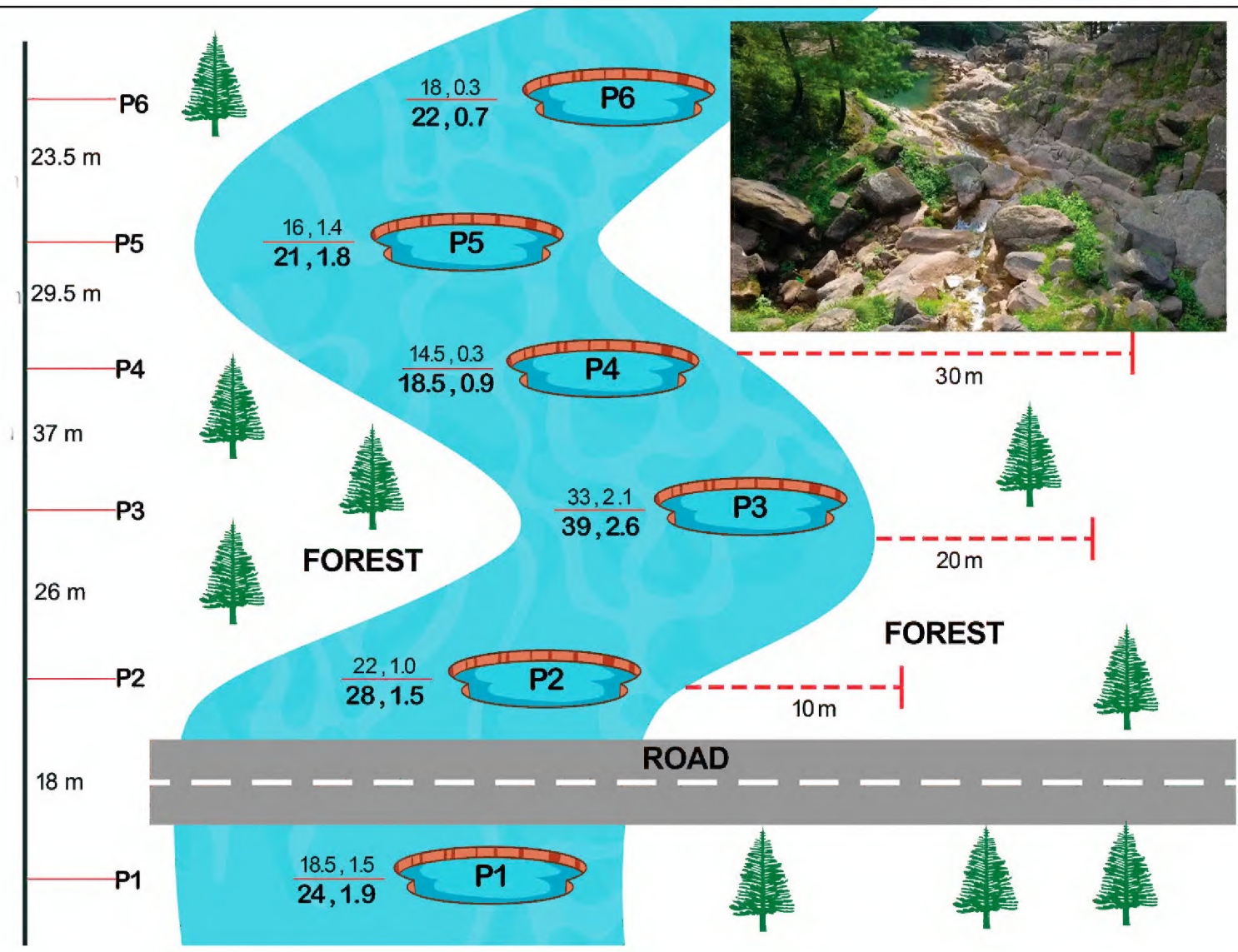

Figure 3. Structure of the study stream (inset photograph shows stream habitat) and its associated ponds. The pond 1 is separated from other ponds by a road. Numerator value shows perimeter m and depth m during pre-breeding season while denominator, in bold, show same measurements for breeding-post-breeding season. The distances (m) between ponds (measured from the center of each pond) are given on the right side. Pre-measured ropes of different lengths (10–30 m) were used to mark distances on either side of the stream.

and recorded the data as described above. The distance moved by the frogs recaptured during this season, which were marked in pre-breeding season, was used to estimate mean distance moved by the frogs for pre-breeding season. We made a two-day visit on 14–15 September, 2019, to estimate the distance moved by the frogs for the breeding-post-breeding season.

We plotted the distance data for the recaptured frogs as box plots for each sex (male and female) and season (pre-breeding, breeding-post-breeding) and ran Wilcoxon test. We also compared (median) distance between species, seasons and sex using Wilcoxon test in R. 4.3.0 ("ggstatsplot") (Patil 2021; R Core Team 2021).

We used Lincoln Index (LI) to estimate the population of each frog species: LI = (N × n) / R where n = number of individuals captured on the first occasion (May, 2019), N = number of individuals captured on the second occasion (August-September, 2019), r = recaptured those with marks (14–15 September, 2019). The standard error (SE) was calculated using formula: √ n2 X N (n-r)/ r3 while the limits of confidence interval (95%) were calculated as 1.96 (SE) ± LI. This is the simplest method based on few episodes of marking and recapturing individuals over a short period of time (Fowler et al. 1998).

Due to financial and technical constraints, we focused on one stream which was selected due to its being a permanent freshwater stream in close proximity to other streams, where both endemic species occurred. The stream is accessible all year round and is safe to carry out nocturnal surveys.

Results

Adult population estimates and sex ratio

We estimated that the number of male and female *N. vicina* was 83 (95% CI: 60–126) and 102 (95% CI: 79–151), respectively and of *A. hazarensis* was 43 (95% CI: 15–97) males and 49 (95% CI: 8–130) females. The number of individuals of *N. vicina* and *A. hazarensis* was estimated to consist of 185 (95% CI: 152–250) and 92 (95% CI: 44–181) individuals from the study area (0.79 ha.) during the study period, respectively. The sex ratio (♂: ♀) of *N. vicina* and *A. hazarensis* was 0.8 (<1):1 and 0.8 (<1):1, respectively (~ 1:1 for both species), during the breeding season.

Daily movement

Pre breeding

The mean distance (pooled for eight days) and the mean daily distance (per day) covered by *N. vicina* (n = 16, Min.–Max.= 0–92 m) during pre-breeding season was 22.71 ± 6.50 m and 2.83 m, respectively. About 25% *N. vicina* moved < 1.25 m (1st quartile) and 75% moved < 29.5 m (3rd quartile). The mean distance (pooled for eight days) and the mean daily distance (per day) covered by *A. hazarensis* (n = 7, Min.–Max.= 0–63 m) was 23.41 ±12.72 m and 2.92 m, respectively. About 25% *A. hazarensis* moved < 0 m and 75% moved < 50.87 m.

Breeding-post-breeding season

The distance (pooled for eight days) and the daily distance (per day) covered by *N. vicina* (n = 3, Min.–Max.= 22–44 m) during breeding-post-breeding season was 33.33 ± 6.35 m and 4.16 m, respectively. About 25% moved < 25 m and 75% moved < 41.5 m. The distance (for eight days) and the mean daily distance (per day) covered by *A. hazarensis* (n = 7, Min.–Max.= 18–81 m) was 51.5 ± 17.10 m and 6.43 m, respectively. About 25% moved < 20 m and 75% moved < 81 m.

Seasonal movement

Pre-breeding

The distance covered by *N. vicina* (n = 11, Min.–Max.= 0–110 m) during the pre-breeding season was 56.86 ± 10.55. About 25% moved < 20 m and 75% moved < 68.5 m. The distance covered by *A. hazarensis* (n = 7, Min.–Max.= 23.5–133.5 m) was 81.66 ± 19.27 m. About 25% moved < 26.62 m and 75% moved < 114.5 m. Four Murree Hills Frog were recorded within 20 m (7, 9, 12 and 18 m) distance outside the stream while only one Hazara Frog was recorded within 10 m (8 m) distance.

Breeding-post-breeding

The distance covered by *N. vicina* (n = 4, Min.–Max. = 22–110 m) during breeding-post-breeding season was 71.75 ± 18.32 m. About 25% moved < 35 m and 75% moved < 102.75 m. The distance covered by *A. hazarensis* (n = 5, Min.–Max.= 0–92.5 m) was 70.1 ± 17.87 m. About 25% moved < 18.5 m and 75% moved < 92 m. Two Murree Hills Frogs were recorded within 20 m (10, 12 m) distance outside the stream while only one Hazara Frog was recorded within 10 m distance. Three Hazara Frog were recorded within 30 m distance (8, 19, 22 m) during September, 2019, recapture period.

The difference between the distance (daily movement) moved by *N. vicina* (sexes pooled) during the two seasons (Fig. 4A) and two sexes (Fig. 4B) and distance (seasonal movement) during the two seasons (Fig. 4C) and two sexes (Fig. 4D) did not differ significantly. The distance (m) moved by the two endemic frogs when species and sexes pooled (Fig. 5A), species pooled (Fig. 5B) and sexes pooled did not differ (Fig. 5C).

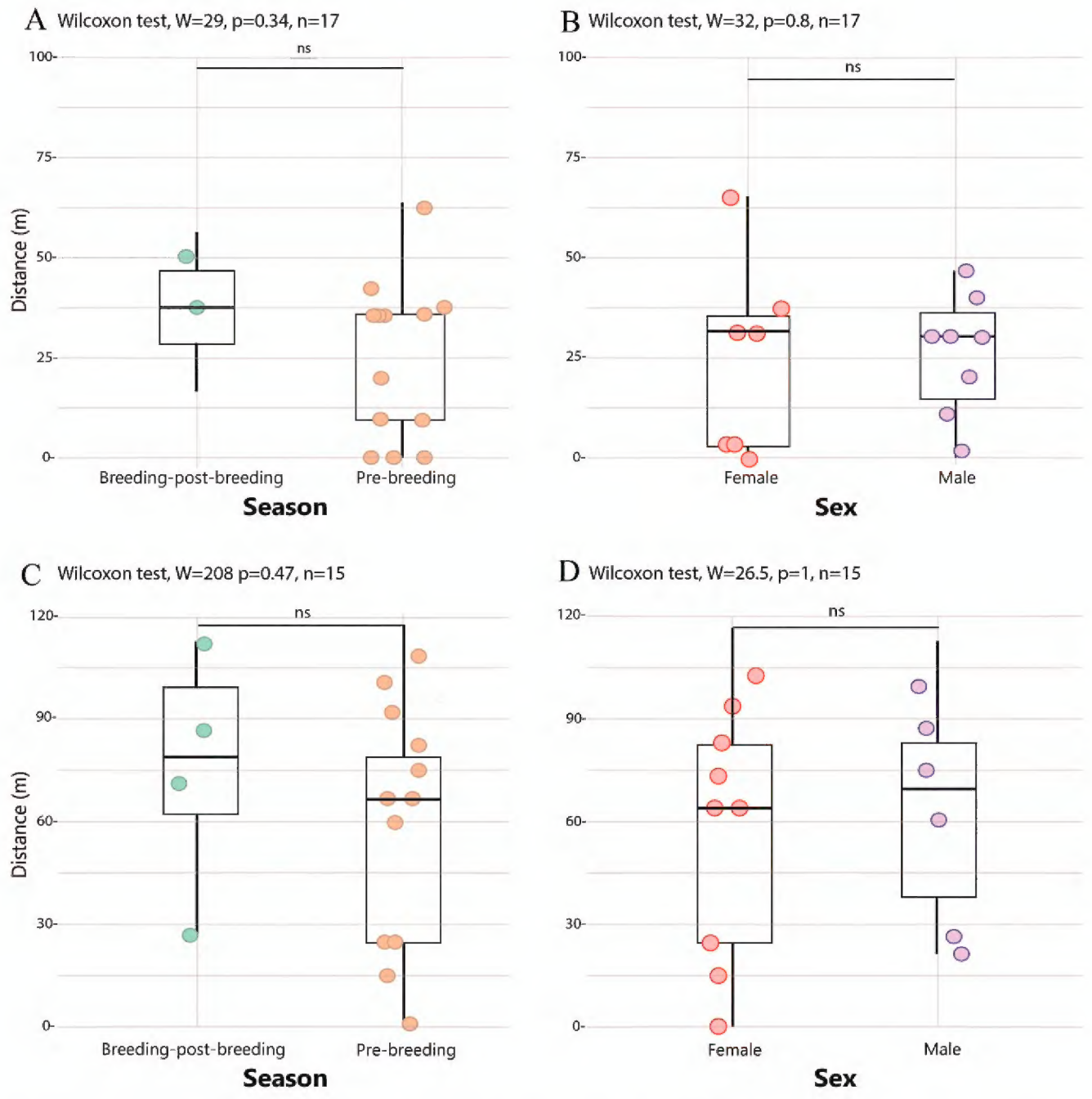

Figure 4. A. Box plot showing comparison between daily distance (m) moved by Murree Hills Frogs (*Nanorana vicina*) during the two seasons (sexes pooled, two 8 days field sessions) (ns= non-significant at α 0.05); **B.** Box plot showing comparison between daily distance (m) moved by males and females of Murree Hills Frog (*Nanorana vicina*) (seasons pooled, two 8 days field sessions) (ns= non-significant at α 0.05); **C.** Box plot showing comparison between seasonal movement (m) exhibited by Murree Hills Frogs (*Nanorana vicina*) during the two seasons (sexes pooled) (ns= non-significant at α 0.05); **D.** Box plot showing comparison between seasonal movement (m) exhibited by males and females of Murree Hills Frog (*Nanorana vicina*) (seasons pooled) (ns= non-significant at α 0.05).

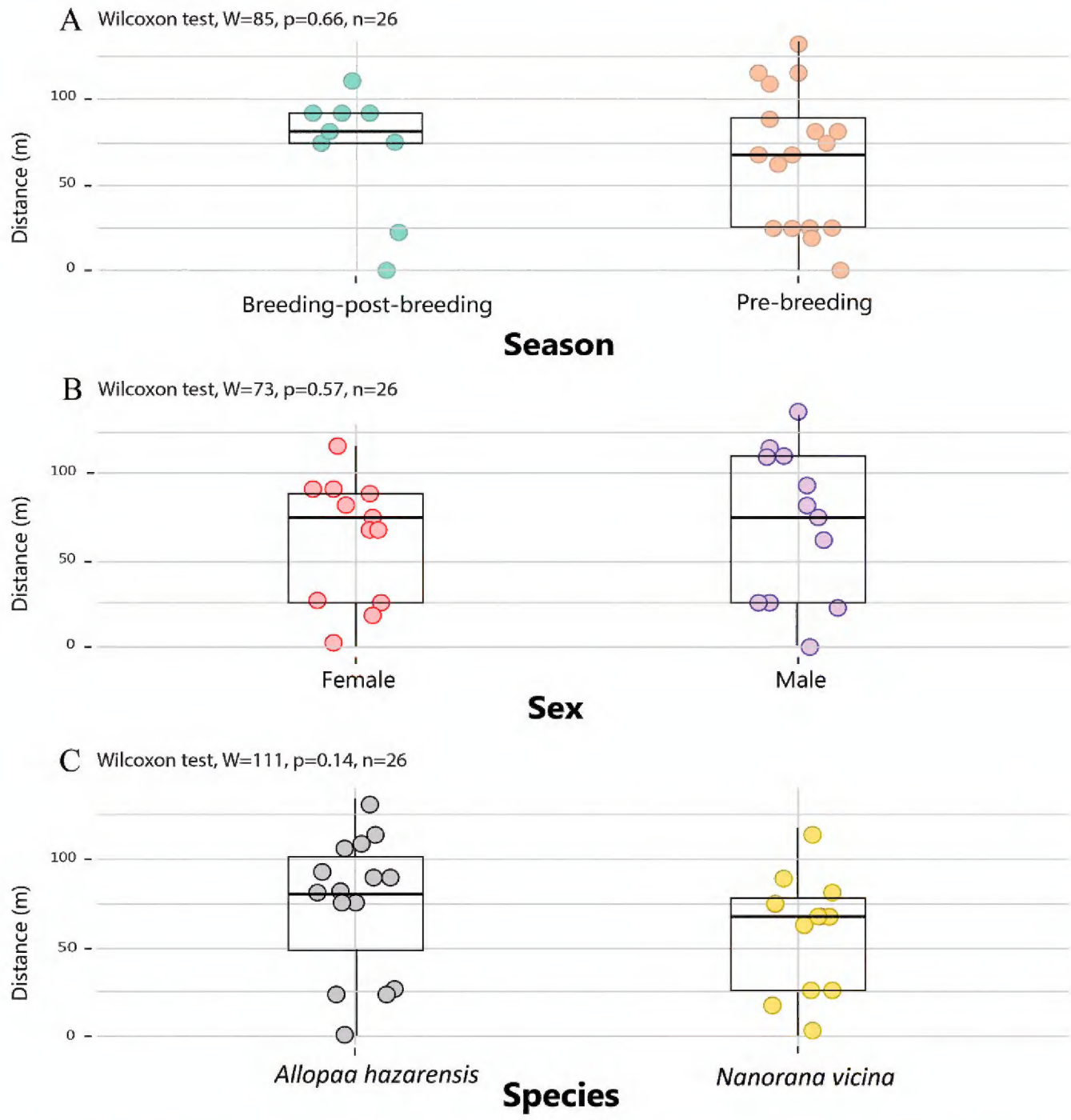

Figure 5. A. Box plot showing comparison between distance (m) moved by the two endemic frogs, Murree Hills Frogs (*Nanorana vicina*) and Hazara Frogs (*Allopaa hazarensis*) (species and sexes pooled), during the pre-breeding and breeding-post-breeding season; **B.** Box plot showing comparison between distance (m) moved by males and females of the two endemic frogs, Murree Hills Frogs (*Nanorana vicina*) and Hazara Frogs (*Allopaa hazarensis*) (species pooled); **C.** Box plot showing comparison between distance (m) moved Murree Hills Frogs (*Nanorana vicina*) and Hazara Frogs (*Allopaa hazarensis*) (sexes pooled).

Discussion

Of the many identified threats globally, habitat degradation, fragmentation, and loss have been recognized as the major factors responsible for the decline of many amphibian species (Brown et al. 2012). This has led to a great deal of research into understanding the ecology of amphibians, particularly their movement and dispersal capabilities, in a landscape undergoing these phenomena. Data from other regions (Nearctic, Palearctic, Neotropical, and Australian region) is available and has greatly increased our understanding of how to incorporate habitat management needs for amphibians into urban planning and development projects. Establishing the negative impact of habitat fragmentation and gathering data on movement and dispersal in amphibian populations is required for properly understanding the situation in the area in question (Funk et al. 2005). We, however, still lack scientific information about species from the Oriental region, particularly from under-developed countries, where resources are limited and smaller wildlife groups, such as amphibians, receive very little attention.

We provide new information about abundance and movement in Asian Spiny Frogs, *N. vicina* and *A. hazarensis*, endemic to Himalayas: Saeed et al. (2022) compared detection of *N. vicina* and *A. hazarensis* using eDNA surveys and visual encounter surveys for estimating occupancy. The former method accounted for higher occurrence probabilities. We report on the population of the two frog species for the first time. The two species were found to be fairly common at the study site. We, however, believe that factors such as open population structure as well as the short period of time that was available for autumn recaptures might affect the accuracy of our abundance estimates.

We found that *N. vicina* and *A. hazarensis* did not exhibit much movement in the two field sessions or during pre- and post-breeding seasons. Movement over short distances in amphibians is common. Of the 53 anuran species reviewed for their dispersal ability, 56% moved a distance of $\leq$ 1 km (Smith and Green 2005). About 30% of the marked individual of *Physalaemus pustulosus* (Tungrana Frog) moved among the ponds and covered a distance of 50 m during the period of five weeks. The

majority of recorded movements were within 200 m distance (Marsh et al. 1999). Houston Toads (*Bufo houstonensis*) stayed within 75 m of the pond of initial capture, rarely dispersing to a distance of > 750 m (Vandewege et al. 2013). Akram et al. (2022) reported that radio tracked Murree Hills Frogs (6 ♂, 7 ♀) and five Hazara Frogs (8 ♂, 8 ♀) remained within 3 m distance during post-breeding (September, 2017 and 2018). The study was, however, conducted during breeding-post breeding season only. The mean daily movement and mean seasonal movement of the studied species were in accordance with the distances known from most amphibian species elsewhere in the world (Marsh et al. 1999; Vandewege et al. 2013).

The movement data, both daily and seasonal, showed that the frogs moved either between neighboring ponds or remained within an area of approximately 120 m^2, which contained a few pools on the river. The maximum linear distance from the breeding pond traveled by 11 studied female *B. americanus* (American Toad) ranged from 246 to 1,015 m (Forester et al. 2006). All males of *B. houstonensis* stayed within < 75 m of their breeding pond (Vandewege et al. 2013). Only 1% (of 10,443 individuals marked) of the adult *Rana luteiventris* (Columbia Spotted Frog) moved ≥ 2000 m among 21 ponds situated within 7 km distance (Funk et al. 2005). About 151 adult Water Frogs (*Rana lessonae*, *R. ridibunda*, and *R. esculenta*) moved among all studied ponds except the one which was separated by a road (Peter 2001). A total of 12 movements were recorded between ponds separated by 50–100 m, seven movements between 150–200 m, six movements of 150–200 m and two movements of > 200 m in *P. pustulosus* over a period of 5 weeks (Marsh et al. 1999). The small distance range in the movements we monitored is attributed to the presence of ponds at different elevations and with differing levels of disturbance. Pond 1 is separated from other ponds by the road, and ponds No. 1 and 2 experience a high level of human disturbance while Pond 5 had the least disturbance. The local community draws water from these ponds for household use, hence, frogs moved from ponds P1 and P2 to P5.

The majority of the movements were along the stream particularly in the upstream direction. The *Ascaphus truei* (Coastal Tailed Frog) is known to move upstream during low water flow in the headwater stream system whilst moving downstream to breed (Hayes et al. 2006). Our studied stream is a permanent freshwater stream. The main source is ground water, snow-melt, and drainage of rainfall from hills located in the surrounding area, especially the northern side. We suggest our species responded to disturbance level and prefer to occupy ponds in the upstream area where there are fewer disturbances.

The study area bears exceptional importance in terms of forest and biodiversity (Chawla et al. 2012). Unfortunately, the area has also been experiencing both natural and human induced changes. About 55 km^2 (24 km^2 in state-owned and 31 km^2 in private or community forest) reduction in the forest area of Tehsil Murree (Rawalpindi, Punjab, Pakistan) is reported (Shahzad et al. 2015). The natural areas have been degraded and the forest area has been transformed by buildings and other urban features. Climate change during the past two decades has affected many areas of Pakistan, including this study area. Over a dozen butterfly species have been lost due to changes in the climate of Murree (Saadat et al. 2016). The two endemic frog species are known to occur in sub-tropical chir pine forest (900–1,700 m) and other forest types such as Himalayan moist temperate forest (> 2000 m) further north of our study area, but do not exist in sub-tropical scrub (broad-leaved) forest (< 900 m) located in the south. The species are adapted to freshwater forested wetlands found at higher elevation with low air and water temperature and cool summers. The long-term survival of many amphibian populations or subpopulations depends on colonization from nearby wetlands (Petranka et al. 2004; Church 2008). There is an inverse relationship between colonization and distance to travel. If an amphibian species is not capable of moving from source pond to other nearby wetlands, the chances of recolonization are expected to decrease (Lehtinen and Galatowitsch 2001) which might cause local extinction. Hence, the presence of wetlands in close proximity helps attain long-term persistence in amphibian populations (Petranka and Holbrook 2006). The two studied species are unable to perform overland migration through the open forest to disperse or colonize nearby streams. Due to various anthropogenic threats or changes in climatic pattern, their movement and dispersal would be limited, resulting in the progressive decline of their populations which might eventually lead to local extinction.

Many researchers have proposed different mitigation approaches such as construction of artificial wetlands (created wetlands) and wetland restoration (Lehtinen and Galatowitsch 2001; Pechmann et al. 2001; Vasconcelos and Calhoun 2004). The natural wetlands, unlike created wetlands, undergo changes in hydrology, water regime, flow and persistence which might impact success of reproduction. Similarly, created wetlands are sometimes free of fish, all potentially negative factors for amphibian reproduction. We suggest inclusion of amphibian habitat requirements and ensuring stream connectivity in urban planning and development projects of the area.

We obtained low recaptures which could be due to the secretive nature of the studied species. These frogs may remain hidden most of the time and it was not possible for us to move the heavy rocks and boulders to check for their presence in these likely locations. The area has been experiencing rapid changes. We recommend carrying out more robust and long-term studies encompassing multiple streams situated within a wider area. This would help to understand the colonization of these frogs and establish if they are capable of moving through the forest to occupy other streams, thus providing a better picture of metapopulation structure and dynamics. Likewise, studies on the impact of water quality and quantity on these frogs would help associate water and amphibian conservation in the area.

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Supplementary material 1

Details of tagging of specimens of *Nanorana vicina* and *Allopaa hazarensis* for pre-breeding season and breeding-post-breeding season

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Data type: table (excel file)

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